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Evaluation of size, morphology, concentration, and surface effect of gold nanoparticles on X-ray attenuation in computed tomography

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ABSTRACT

Increasing attention has been focused on the use of nanostructures as contrast enhancement agents in medical imaging, especially in computed tomography (CT). To date, gold nanoparticles (GNPs) have been demonstrated to have great potential as contrast agents for CT imaging. This study was designed to evaluate any effect on X-ray attenuation that might result from employing GNPs with a variety of shapes, sizes, surface chemistries, and concentrations. Gold nanorods (GNRs) and spherical GNPs were synthesized for this application. X-ray attenuation was quantified by Hounsfield unit (HU) in CT. Our findings indicated that smaller spherical GNPs (13 nm) had higher X-ray attenuation than larger ones (60 nm) and GNRs with larger aspect ratio exhibited great effect on X-ray attenuation. Moreover, poly ethylene glycol (PEG) coating on GNRs declined X-ray attenuation as a result of limiting the aggregation of GNRs. We observed X-ray attenuation increased when mass concentration of GNPs was elevated. Overall, smaller spherical GNPs can be suggested as a better alternative to Omnipaque, a good contrast agent for CT imaging. This data can be also considered for the application of gold nanostructures in radiation dose enhancement where nanoparticles with high X-ray attenuation are applied.

1. Introduction

Computed tomography (CT) is considered as one of the most useful diagnostic tools for evaluating approximately all organs in modern medicine owing to its low cost, wide viability, good deep tissue penetration, efficiency, as well as better spatial and density resolution than other imaging devices [1–4]. To enhance contrast resolution, iodine-containing molecules are most generally used as CT contrast agents, due to their higher X-ray attenuation than normal tissue. Iodinated molecules have an approximately short blood circulation time and rapid clearance from the kidneys. The search for an optimal CT contrast agent with maximum imaging capabilities, reduced toxicity, and minimal dose requirements are essential [5–8]. In the design of a CT contrast agent, several common requirements should be considered: 1) it should be able to make better visualization of the target tissue by increasing the CT attenuation difference between the target and surrounding tissues; 2) it should have adequate concentrations required for imaging and be nontoxic and biocompatible; and 3) it must have a long circulation time to be enough for good efficiency and timing in CT scan imaging [9–11]. Recently, a wide spectrum of materials and contrast agent designs have been investigated in both clinical and laboratory settings. GNPs have a strong chemical, physical, and biological confidence. Good biocompatibility, long circulation time, and ease of surface modification make GNPs promising nanoprobes for diagnostic imaging [12–15]. The density and atomic number of gold (19.32 g/cm^3 and 79, respectively) are much higher than those of the presently used iodine (4.9 g/cm^3 and 53). The photon attenuation coefficients of gold and iodine at 100 keV are 5.16 and 1.94 respectively; therefore, gold has 2.7 times higher contrast per unit mass than iodine [10,16] in X-ray imaging. Monte Carlo simulations demonstrated that spectral CT imaging of liver lesions with GNPs is feasible and the simulations revealed the possibility of spectral CT imaging with CNRs of the particular gold signal between 2.7 and 4.8 after bilateral filtering [17]. For CT imaging of biological systems, appropriate GNPs are needed to progress diagnostic precision, which has become an important challenge for physicians and researchers. It is important and beneficial to study whether

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various parameters of GNPs could be utilized to improve contrast enhancement. The size and morphology of GNPs are well known to affect the scattering and absorption of visible light, which enticed researchers to investigate similar effects on X-ray scattering and absorption [18,19]. Absorption of X-ray radiation by high z number elements like gold has been recently investigated and reported. The contribution of GNPs in increasing the radiotherapy efficiency is measured by the sensitizer enhancement ratio (SER) or dose enhancement factor (DEF). Some studies have demonstrated that the dose to tissue volumes can vary with the characteristics of the GNPs and concentration. Studies show that combination of the GNPs with clinical 4 MeV electron beams could enhance the effect of radiotherapy. GNPs exhibit maximum enhanced secondary electron ratio at a size of 2 nm and the effect is decreased by increasing the size of GNPs [20–22]. To the best of our knowledge, no study has simultaneously investigated the effects of GNPs particle size, morphology, surface, and concentration on the attenuation value for polychromatic X-ray beam in CT imaging. Thus, we aimed to compare several samples of GNPs suspensions, each with the same concentration but with a large disparity in mean particle size, morphology, and surface. The distribution of pharmaceutical nanoparticles is investigated by a variety of pharmacological and physiological factors. Based on Moghimi and Hamad, the path of these materials in the body can be affected by shape, size, density, surface characteristics, biological ligands, and polymer type of nanoparticles [23]. GNPs can also affect cellular uptake and circulation time. GNPs with mean diameter of 100 nm exhibited greater retention in the blood compared with 10, 50, and 250 nm GNPs after 24 h in rats [24]. However, another study reported than gold nanoparticles of 15 nm and 50 nm sizes exhibited longer blood circulation than particles of 100 nm and 200 nm sizes, in mice [25]. Surface functionalization of GNPs with poly (ethylene glycol) (PEG) has been widely evaluated. Compared to bare GNPs, PEGylated GNPs show longer blood half-life in vivo [26]. Rapid blood clearance can undermine the efficiency and mass concentration that is able to attain the site of interest. PEG chain length can affect half-life; for instance, 18 nm GNPs coated with 2 kDa PEG molecules revealed a half-life of nearly 4 h, while with 10 kDa PEG molecules they showed a half-life of approximately 51 h [25,26]. In addition to the effect of size and morphology of GNPs on X-ray attenuation, we sought to clarify how modifications of GNPs surface can impact X-ray attenuation. This is the first attempt to simultaneously evaluate the impact of these factors with different kVps and concentrations on X-ray attenuation.

2. Materials and methods

2.1. Materials

Water was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity of 18.2 MΩ cm.

2.2. Spherical GNPs synthesis

The spherical particles were synthesized through chemical reduction of Au^{+} ions with citrate ions under reflux condition [27]. The reduction of a hydrogen tetrachloroaurate (III) trihydrate ($HAucl_4{\cdot}3H_2O$) solution (Alfa Aesar, U.S.A) was started by tri-Sodium citrate (Merck, Germany) by reaching the gold solution to the boiling temperature in oil. When the solution (100 mL) started to boil, 1 mL of citrate solution was added. The citrate concentration was varied to gain various particle sizes. After 15 min, the liquid was cooled to room temperature.

2.3. Synthesis of cetyltrimethylammonium bromide (CTAB)-stabilized GNRs

Nanorods were prepared via seed mediated method [28]. To prepare a gold seed solution, in a typical method, 0.250 mL of an aqueous 0.01 M solution of hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4{\cdot}3H_2O$) was added to 7.5 mL of a 0.10 M CTAB solution. Then, 0.600 mL of an aqueous 0.01 M ice-cold sodium borohydride ($NaBH_4$) (Merck, Germany) solution was quickly added all at once, followed by rapid stirring for 2 min and then kept at 25 °C for future use.

Separately, in a typical experiment a gold growth solution was prepared by adding 0.20 mL of 0.01 M $HAuCl_4{\cdot}3H_2O$ to 4.75 mL of 0.1 M CTAB. Afterwards, 0.03 mL of 0.01 M silver nitrate ($AgNO_3$) (Merck, Germany) was added. The mixture at this step appeared bright brown-yellow in color. Then, 0.032 mL of 0.1 M ascorbic acid (AA) (Merck, Germany) was added to it. Finally, 0.01 mL of seed solution was added and then the mixture was gently mixed for 10 s and left for at least 3 h.

2.4. Synthesis of PEG-stabilized GNRs

To remove unbound CTAB, before functionalization with thiolated polyethylene glycol ($mPEG_{6000}$-SH), GNRs were centrifuged twice at 12,000 rpm for 12 min. Thereafter, 5 mL of mPEG-SH was added to GNRs and put in an incubator shaker for 24 h at 30 °C. Ultimately, to ensure removing all CTAB, the mixture was dialyzed through a 14 kDa membrane against ultrapure water for 48 h.

2.5. Nanoparticle characterization

Transmission electron microscopy (TEM) was performed (Zeiss EM 900, Germany) to investigate morphology and size of the GNPs. Optical spectra of GNPs were recorded by using a SPEKOL 2000 (Analytik Jena AG, UK) spectrophotometer. The synthesized GNRs had two plasmon peaks, a transverse plasmon peak, and a longitudinal plasmon peak. The concentrations of GNPs in μg/ml were measured by the inductively coupled plasma optical emission spectrometry (ICP-OES). Chemical component was also determined by a Raman Spectrometer (Avantes, Netherlands).

2.6. X-ray attenuation measurements

GNPs and Omnipaque solutions with different concentrations, ranging from 100 to 400 μg/ml, were prepared in 0.5 mL Eppendorf Tubes and placed in a homemade poly methyl methacrylate (PMMA) phantom. The phantom was scanned using a CT imaging system (GE Light Speed VCT 64 slice CT scanner) with 80, 100, 120, 140 kVp, 200 mAs, and a slice thickness of 0.625 mm. In order to evaluate the images in terms of GNP X-ray attenuation, all the acquired images were loaded to a standard program (DICOM reader). Uniform ellipse region of interest (ROI) with dimensions of about 24 pixels (0.032 cm^2) were placed on each sample inside CT images. The Hounsfield units (HU) for each ROI containing types of GNPs and Omnipaque (general electric Healthcare Ireland, Cork, Ireland) were investigated.

2.7. Statistical analysis

All the data was compared for significant difference in X-ray attenuation by One-way analysis of variance and Tukey's multiple comparison tests. P-value less than .05 was considered statistically significant.

3. Results

3.1. Synthesis and characterization of GNPs and GNRs

GNP suspensions were synthesized by the previously delineated protocols. TEM images and UV-visible spectra are presented in Fig. 1. Synthesis methods were successful in preparing a wide range of particle sizes. Formation of spherical GNPs and GNRs were confirmed by appearance of typical surface plasmon resonance in the UV-visible